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13. ABSTRACT *(Maximum 200 Words)*

Orphan nuclear receptor COUP-TFII plays important roles in controlling diverse aspects of cell growth, development, differentiation and homeostasis. During the development, its expression is spatially and temporally restricted within mesenchymal cells of many organs that require interactions between the mesenchymal and epithelial compartments for proper development. To study the possible role of COUP-TFII during the development of prostate, we have investigated the expression pattern of COUPTFII in mouse male reproductive organ at different stages by using *in situ* hybridization. Our results showed that COUPTFII is highly expressed in mesenchymal cells surrounding ventral prostate, dorsal lateral prostate, vas deferens and other male reproductive organs, suggesting that COUP-TFII may be an important modulator for mesenchymal-epithelial interactions required for the normal prostate development and tumorgenesis of prostate cancer. To further study COUP-TFII's function during the carcinogenesis of prostate cancer, we have successfully generated adenoviral vectors that effectively express sense and antisense COUP-TFII mRNA. Adenovirus-infected prostate cells or control cells were then introduced into asthymic nude mice to analyze the effect of COUP-TFII over- or under-expression on the ability of tumorigenesis of prostate cancer cell lines. For unknown reason, however, primary results were inconsistent and inconclusive. We are now investigating possible reasons and evaluating feasible solutions.

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Introduction

Mesenchymal-epithelial interactions play pivotal roles in the regulation of growth and function of prostate epithelial cells. Prostate epithelial differentiation is dictated by its surrounding mesenchymal cells, which determine androgen induced growth responsiveness and the expression of specific secretory proteins in normal prostate gland. During neoplastic progression, organ specific mesenchymal cells have been shown to determine the rate of prostate tumor growth, differentiation and androgen responsiveness (1-4). These indicate the importance of the paracrine signal(s) produced by mesenchyme in determining the cell fate and differentiation of nearby prostate epithelial cells. Orphan nuclear receptor COUP-TFII, a transcription factor that belongs to the steroid/thyroid hormone receptor superfamily, plays important roles in controlling diverse aspects of cell growth, development, differentiation and homeostasis. During the development, its expression is spatially and temporally restricted within mesenchymal cells of many organs that require interactions between the mesenchymal and epithelial compartments for proper development. We have systematically studied the COUP-TFII expression pattern in mouse reproductive organ during the development by section *in situ* hybridization. Our results showed that COUP-TFII is expressed in mesenchymal cells surrounding prostate epithelium during early prostate development. This strongly suggests that COUP-TFII may be an important modulator not only for mesenchymal-epithelial interactions, but also for normal prostate development as well as tumorgenesis of prostate cancer. To further study COUP-TFII's function during the development of prostate gland and carcinogenesis of prostate cancer, we propose to investigate the effect of COUP-TFII over- and under-expression on the ability of mesenchyme to induce epithelial differentiation in kidney capsule, and the growth rate of prostate tumors induced by epithelial cells in athymic nude mice. This study will provide us important insights for possible roles of COUP-TFII during the development of the prostate and the tumor progression of prostate cancer. It may raise the possibility that COUP-TFII and its regulated-signal molecules could be used as targets to establish novel therapeutic strategies for the improvement of diagnosis, prevention, prognosis and treatment of human prostate cancer and other forms of malignancies.

Specific Aims:

Aim 1. Effect of COUP-TF over- or under-expression on the ability of mesenchyme to induce epithelial differentiation in kidney capsule. We will construct adenoviral vectors capable of over-expressing or under-expressing COUP-TFII by expressing sense or antisense COUP-TFII messenger RNA. These adenovirus expression vectors will be used to express COUP-TFII in isolated primary mesenchymal cells from the urogenital sinus. The resulting cells will then be reconstituted with isolated prostate epithelial cells in the kidney capsule to study their effect on prostate epithelial differentiation.

Aim 2. Effect of over- and under-expression of COUP-TFII on the growth rate of prostate tumors induced by epithelial cells in nude mice We will develop vectors capable of over-expressing or under-expressing COUP-TFII by expressing sense, antisense, or dominant-negative COUP-TFII messenger RNA, and generate stable rUGM cell lines with these constructs. Once these cell lines are established, we will introduce them together with prostate epithelial cells into male nude mice and assess the effect of COUP-TFII expression on the growth rate and differentiation of resulting tumors.

Research Accomplishments:

Study of COUP-TFII Expression Pattern during Development of Mouse Male Reproductive Organ

To investigate the possible role of COUP-TFII in prostate development, we first analyze its expression pattern in mouse male reproductive tract during the development by section *in situ* hybridization. By 17.5 dpc, when the prostatic buds start to arise as outbuddings of the urogenital sinus epithelium into the surrounding mesenchyme to form prostate gland, COUP-TFII transcripts were detected in urogenital sinus mesenchymal

cells surrounding anterior prostate bud, Wolffian duct-derived seminal vesicles and vas deferens (Fig. 1). Postnatally, COUP-TFII is continuously expressed at high levels in mesenchymes surrounding ventral prostate, dorsallateral prostate, and vas deferens (Fig. 2). We also examined the distribution of COUP-TFII transcripts in male reproductive tract in the adult mouse. We found by ribonuclease protection analysis that COUP-TFII expression was detected in ventral, dorsallateral prostates, vas deferens, epididymus, and seminal vesicles (Fig. 3).

Effect of over- and under-expression of COUP-TFII on the growth rate of prostate tumors induced by epithelial cells in nude mice

Signaling interactions between epithelium and mesenchyme are required for normal prostate growth and differentiation while deranged interactions may contribute to the inappropriate reactivation of cellular proliferation that occurs during aging (5-6). During embryogenesis, inductive signals from the urogenital sinus mesenchyme induce the adjacent epithelium to form prostatic buds (7-8). Postnatally, reciprocal interactions between epitheliaum and mesenchyme are also required for ductal morphogenesis and prostate maturation (9). Our results showed that COUP-TFII is expressed in mesenchymal cells surrounding prostate epithelium during early prostate development, strongly suggesting that COUP-TFII may be an important modulator not only for normal prostate development, but also for tumorgenesis of prostate cancer. To test this hypothesis, we have successfully generated adenovirus that expressed sense or antisense COUP-TFII (last year annual summary). The adenovirus-infected prostate cells DU145 were then injected into nude mice to evaluate the effect of over- and under-expression of COUP-TFII on the growth rate of prostate tumors induced by DU145 cells.

Initially, our results from the first set experiment were quite encouraging (Table 1). 6 weeks after injection, we found 7 tumors out of 8 injected points in our control group (no adenovirus-infected DU145 cells), 7 tumors out of 12 injected points in the group of COUP-TFII sense virus, and 0 tumor out of 12 injected points in the group of COUP-TFII antisense virus. These data suggested that antisense COUP-TFII maybe inhibit tumor formation induced by DU145 cells in nude mice. However, in subsequent there

independent experiments, our data are inconsistent and inconclusive, which are elaborated in following:

Set A (Table 2): Within first 3 weeks of injection, it seemed that antisense group inhibited tumor growth, and sense group promoted tumor formation. However, after 3 weeks, both sense group's and some of control group's tumors started to regress, which makes us hard to make a judgment.

Set B (Table 3): Tumors in both sense and antisense group regressed, indicating some toxicity of virus to infected DU145 cells.

Set C (Table 4): Similar results as that from Set B experiment, tumors in both sense and antisense group regressed after two weeks of injection. The data is inconclusive.

Potential Problems

The inconclusive results from our tumor induction experiments in nude mice are probably due to the toxicity of adenovirus that inhibits cell growth in mice. The reasons for this problem are still unknown. We used virus titer was 10^7 to 10^9 particles/ml which showed no problem in transient transfection assay (last year summary) and were successfully used by other researchers in our lab. (10). One possibility could be that virus was contaminated with toxic materials such as endotoxin during virus preparation. To avoid this, we are trying to isolate new batch of virus or different purification protocols. Certainly, we also have to realize the possibility that our strategy may not be right or the nature of COUP-TFII which maybe influence cell growth in both under-expression and over-expression situations.

Because of problems we are facing, we feel uncomfortable to conduct experiments to study the effect of COUP-TF over- or under-expression on the ability of mesenchyme to induce epithelial differentiation in kidney capsule as we originally proposed at this moment.

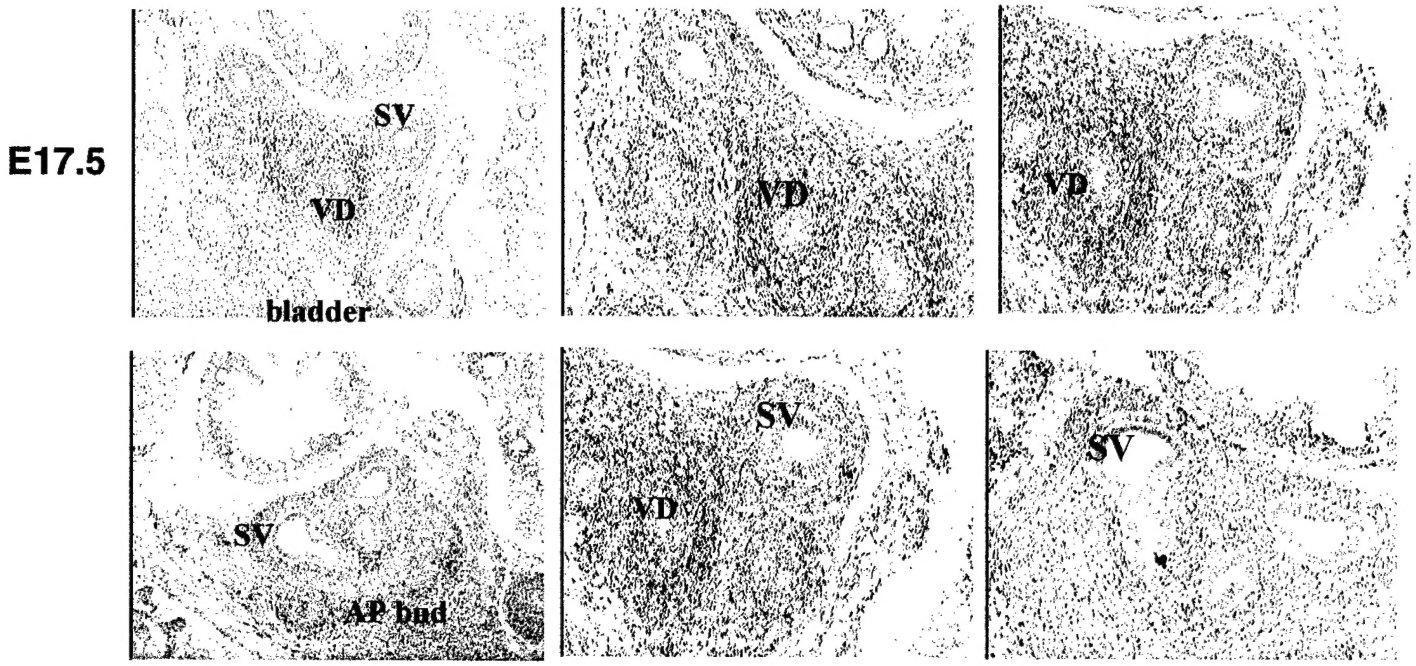
Figure Legend:

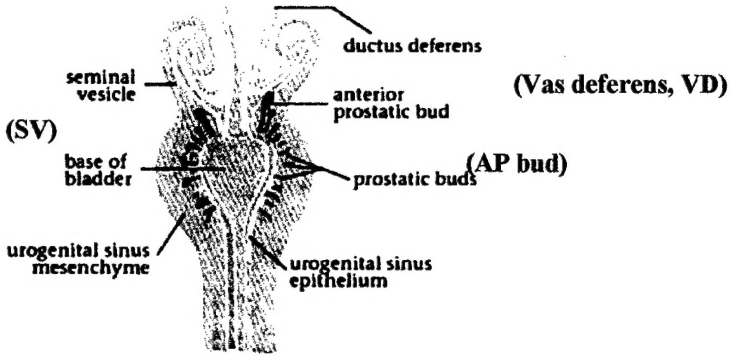
FIG. 1: COUP-TFII expression in male urogenital tract during embryonic development. Transverse sections of urogenital tract were hybridized with a S^{35}-labelled riboprobe of COUP-TFII and counterstained with hematoxylin. Dark-grey particles represent positive signal.

FIG. 2: COUP-TFII expression in male urogenital tract during postnatal development. Transverse sections of urogenital tract were hybridized with a ^{35}S-labelled riboprobe of COUP-TFII and counterstained with hematoxylin. The upper panels are the brightfield views. The bottom panels are the darkfield views colored with red and superimposed upon the brightfield images. Abbreviations: DLP, dorsolateral prostate; U, urethra; VD, vas deferens; VP, ventral prostate.

FIG. 3: Ribonuclease protection analysis using total RNA (20 μg) from the indicated tissues of adult (8-week) male mice, using a COUP-TFII antisense riboprobe. The cyclophilin riboprobe serves as an internal control for RNA loading. : DLP, dorsolateral prostate; U, urethra; VD, vas deferens; VP, ventral prostate; AP, anterior prostate; SV, seminal vesicle; ED, epidydimis.

Expression of COUP-TFII in male reproductive organs





Neonate
(P0, ventral view)

Figure 1

COUP-TFII Expression in Male Urogenital Tract

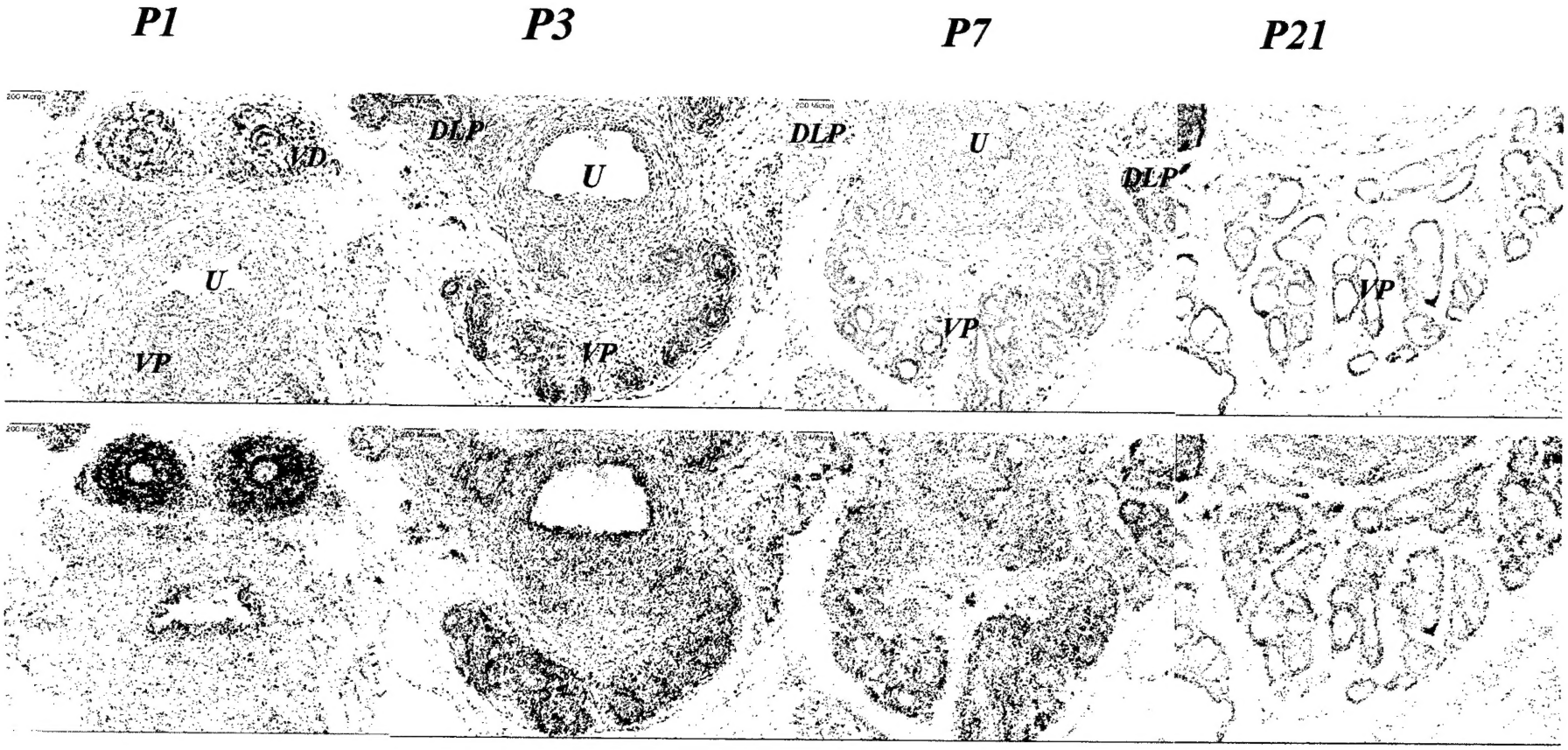


Figure 2

COUP-TFII expression by RPA

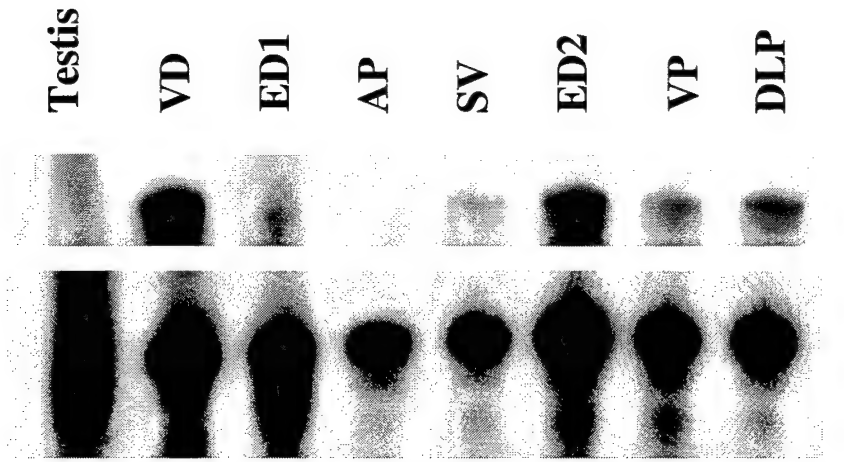


Figure 3

Table 1. Summary of data from Jun 30 to Aug 14 Experiment

	Tumor/Total Injection	
	30 days	42 days
Control	7/8	7/8
Antisense	0/12	0/12
Sense	5/12	7/12

A set 7 days		tumor size	
tumor No.	control	Antisense	sense
1	18	4	13.5
2	18	0	18
3	13.5	4	18
4	13.5	0	13.5
5	13.5	0	4
6	4	4	13.5
7	18	4	18
8	13.5	0	4
9	4	0	0
10	13.5	0	13.5
11	13.5	0	4
12	4	0	13.5

A set 14 days		tumor size	
tumor No.	control	Antisense	sense
1	18	13.5	60
2	18	0	75
3	18	13.5	87.5
4	13.5	0	40
5	13.5	0	4
6	0	13.5	24
7	18	13.5	40
8	0	0	13.5
9	13.5	0	0
10	18	13.5	18
11	18	0	4
12	13.5	0	18

A set 24 days		tumor size	
tumor No.	control	Antisense	sense
1	32	0	60
2	24	0	40
3	24	0	60
4	0	0	32
5	0	0	0
6	0	0	13.5
7	18	0	18
8	0	0	0
9	0	0	0
10	13.5	0	13.5
11	13.5	0	0
12	0	0	0

A set 31days		tumor size	
tumor No.	control	Antisense	sense
1	84	0	40
2	30	0	24
3	40	0	40
4	30	0	24
5	18	0	0
6	0	0	0
7	18	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0

A set 41days		tumor size	
tumor No.	control	Antisense	sense
1	168	0	40
2	60	0	0
3	140	0	24
4	60	0	18
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	50	0	0
10	0	0	0
11	50	0	0
12	0	0	0

Table 2. Data from set A experiment

B set 11 days			
		tumor size	
tumor No.	control	Antisense	sense
1	24	0	18
2	24	13.5	18
3	24	0	13.5
4	13.5	13.5	18
5	13.5	0	13.5
6	0	13.5	0
7	13.5	13.5	18
8	13.5	0	13.5
9	18	13.5	18
10	13.5	0	18
11	13.5	13.5	13.5
12	13.5	13.5	0

B set 18 days			
		tumor size	
tumor No.	control	Antisense	sense
1	60	0	13.5
2	13.5	0	13.5
3	40	0	24
4	0	0	13.5
5	40	0	13.5
6	0	0	0
7	24	0	13.5
8	30	0	13.5
9	40	0	13.5
10	4	0	13.5
11	32	0	0
12	18	0	0

B set 28 days			
		tumor size	
tumor No.	control	Antisense	sense
1	105	0	0
2	0	0	0
3	60	0	13.5
4	0	0	0
5	60	0	0
6	13.5	0	0
7	40	0	0
8	40	0	0
9	60	0	0
10	0	0	0
11	50	0	0
12	0	0	0

B set 36 days			
		tumor size	
tumor No.	control	Antisense	sense
1	168	0	0
2	75	0	0
3	75	0	0
4	50	0	0
5	168	0	0
6	60	0	0
7	87.5	0	0
8	40	0	0
9	100	0	0
10	50	0	0
11	252	0	0
12	50	0	0

B set 43 days			
		tumor size	
tumor No.	control	Antisense	sense
1	540	0	0
2	168	0	0
3	440	0	0
4	60	0	0
5	280	0	0
6	105	0	0
7	252	0	0
8	168	0	0
9	385	0	0
10	75	0	0
11	280	0	0
12	105	0	0

Table 3. Data from set B experiment

C set 7 days		tumor size	
tumor No.	control	Antisense	sense
1	40	24	40
2	24	13.5	0
3	30	18	13.5
4	18	13.5	0
5	40	0	24
6	24	0	13.5
7	40	0	0
8	24	13.5	0
9	40	18	13.5
10	13.5	18	0
11	30	13.5	18
12	18	13.5	13.5

C set 15 days		tumor size	
tumor No.	control	Antisense	sense
1	30	0	18
2	24	0	13.5
3	24	0	0
4	13.5	0	13.5
5	13.5	0	13.5
6	0	0	0
7	13.5	18	13.5
8	0	0	0
9	18	13.5	0
10	0	0	0
11	13.5	13.5	18
12	13.5	0	0

C set 22 days		tumor size	
tumor No.	control	Antisense	sense
1	40	0	0
2	30	0	0
3	30	0	0
4	24	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	40	0	0
10	0	0	0
11	30	0	0
12	13.5	0	0

Table 4. Data from set C experiment

Reportable Outcomes

1. We generated adenovirus which express sense and antisense *COUP-TFII*;
2. We established rat urogenital mesenchymal cell lines which over- and under-express *COUP-TFII* mRNA;
3. Because of the support of this award, I got employment and research opportunities as a Postdoc fellow in Dr. Sophia Tsai's group at Baylor College of Medicine during past two years, which greatly enriched my personal and professional experience.

Conclusions

As summarized above, we have systematically studied the COUP-TFII expression pattern in mouse reproductive organ during the development by section *in situ* hybridization. Our results showed that COUP-TFII is expressed in mesenchymal cells surrounding prostate epithelium during early prostate development. We have successfully generated adenoviral vectors that effectively express sense and antisense COUP-TFII. Adenovirus-infected prostate cells or control cells were then introduced into nude mice to analyze the effect of COUP-TFII over- or under-expression on the ability of tumorigenesis of prostate cancer cell lines. For unknown reason, our primary results were inconsistent and inconclusive. We are now investigating possible reasons and evaluating feasible solutions.

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Appendices

Key research accomplishment:

1. We studied COUP-TFII expression pattern in male urogenital tract during embryonic and pastnatal development;
2. We generated adenovirus that express sense and antisense COUP-TFII, and tested the effect of over- and under-expression of COUP-TFII on the growth rate of prostate tumors induced by epithelial cells in nude mice